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Isolation and Prevalence of *Campylobacter* in the Reproductive Tracts and Semen of Commercial Turkeys

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SUMMARY. *Campylobacter* is one of the most commonly reported bacterial causes of human foodborne infections in the United States, and epidemiologic evidence indicates that a significant proportion of human infections result from the improper preparation of poultry products. *Campylobacter* frequently colonizes the avian intestinal tract, but recent research indicates that this organism can also colonize the avian reproductive tract and possibly contaminate eggs and subsequent offspring. The present studies were undertaken to determine the prevalence of *Campylobacter* in the reproductive systems of commercial turkeys. In the first study, pooled semen samples from seven commercial turkey farms were randomly collected by abdominal massage over a period of 13 wk. The pooled semen samples were serially diluted, and 0.1 ml of each dilution was plated on *Campy*-Line agar and incubated at 42 C for 48 hr in a microaerophilic environment for enumeration of *Campylobacter*. *Campylobacter* was isolated from 57 of the 59 pooled semen samples, and levels ranged from below the limit of detection ($<10^1$) to 1.6×10^6 cfu/ml of semen. In the second study, the reproductive tracts of 11 hens and 17 toms were aseptically excised, and the segments (female: vagina, shell gland, isthmus, magnum, and infundibulum; male: ductus deferens and testes) were swabbed with a dry cotton sterile swab. The swabs were incubated for 24 hr in *Campylobacter* enrichment broth, and 0.1 ml of the enriched sample solution was streaked onto *Campy*-Line agar plates and incubated at 42 C for 48 hr in a microaerophilic environment. Of the 11 hens sampled, *Campylobacter* was isolated from the vagina (10/11), the shell gland (7/11), the isthmus (8/11), the magnum (6/11), and the infundibulum (4/11). Of the 17 toms sampled, *Campylobacter* was isolated from the ductus deferens (8/17) and the testes (2/17). *Campylobacter* is present in the reproductive tracts and semen of commercial turkeys and may lead to vertical transmission of *Campylobacter* from the hen to the chick.

RESUMEN. Aislamiento y prevalencia de *Campylobacter* en los tractos reproductivos y semen de pavos comerciales.

Campylobacter es una de las causas de infecciones asociadas con alimentos que se reporta con mayor frecuencia en los Estados Unidos y la evidencia epidemiológica indica que una proporción importante de las infecciones en humanos resulta de la preparación inadecuada de productos avícolas. El *Campylobacter* frecuentemente coloniza el tracto intestinal, pero una investigación reciente indica que este organismo puede colonizar también el tracto reproductivo y posiblemente contaminar huevos y a la progenie. El presente estudio se realizó para determinar la prevalencia de *Campylobacter* en el sistema reproductivo de pavos comerciales. En el primer estudio, usando masaje abdominal, se tomaron aleatoriamente durante un periodo de 13 semanas, mezclas de muestras de semen de siete granjas de pavos comerciales,. Estas muestras se diluyeron de manera serial y 0.1 ml de cada dilución se sembró en placas de agar Campy-Line y fueron incubadas a 42 °C por 48 horas en un ambiente microaerofílico para recuento de colonias de *Campylobacter*. El *Campylobacter* fue aislado de 57 de las 59 muestras de semen mezcladas y los niveles estuvieron en un rango por debajo del límite de detección ($<10^1$) a 1.6×10^6 unidades formadoras de colonias por mililitro de semen. En el segundo estudio, los tractos reproductivos de 11 gallinas y 17 machos fueron extraídos asépticamente y los segmentos (del tracto femenino: vagina, útero, istmo, magno e infundíbulo y por parte del masculino: conductos deferentes y testículos) fueron muestreados mediante hisopos de algodón estériles. Las muestras de hisopos fueron incubadas durante 24 horas en caldo de enriquecimiento para

Campylobacter y 0.1 ml de la muestra enriquecida fue sembrada en placas de agar Campy-Line e incubadas a 42 °C por 48 horas en ambiente microaerofílico. De las once hembras muestreadas, se aisló *Campylobacter* de la vagina (10/11), útero (7/11), istmo (8/11), magno (6/11) y del infundíbulo (4/11). De los 17 machos muestreados, se aisló *Campylobacter* de los conductos deferentes (8/17) y de los testículos (2/17). El *Campylobacter* estuvo presente en el tracto reproductivo y semen de pavos comerciales y puede provocar la transmisión vertical de *Campylobacter* de la gallina al pollo.

Key words: *Campylobacter*, turkeys, reproductive tract, semen

Abbreviations: C = *Campylobacter*; CEB = *Campylobacter* enrichment broth; CLA = *Campy*-Line agar

Campylobacter is one of the most commonly reported bacterial causes of human foodborne infections in the United States (8,17), with an estimated 2.1 to 2.4 million cases reported annually (2). Epidemiologic evidence indicates that a significant proportion of human infections result from the improper preparation of poultry products (10,21). Numerous studies have shown that a substantial number of retail chicken and turkey products are contaminated with *Campylobacter* (19,25).

It is known that *Campylobacter* frequently colonizes the avian intestinal tract (1,3), and many studies indicate that horizontal transmission from environmental sources is the primary route of infection (20,29). Recent research, however, has demonstrated that *Campylobacter* can also colonize the avian reproductive tract (4,6,14) and may be vertically transferred between broiler breeder flocks and their offspring (11). In addition, these researchers reported that *Campylobacter* was present in the semen and ductus deferens of commercial broiler breeder roosters (12,13).

Semen on commercial turkey farms is routinely pooled and then used to inseminate multiple hens (15) and therefore may be a critical source of *Campylobacter* contamination in turkeys. In a recent study, *Campylobacter* was detected in pooled semen samples of commercial turkeys (16). This initial study detected *Campylobacter* in pooled semen samples but did not evaluate the incidence or concentration of the organism in semen from numerous farms (16). Furthermore, to our knowledge, the presence of *Campylobacter* in the reproductive tract has not been evaluated in turkeys. Therefore, the present studies were undertaken to 1) evaluate the prevalence and concentration of *Campylobacter* in the semen of commercial toms and 2) to determine if naturally occurring *Campylobacter* could be isolated in the reproductive tracts of commercial breeder turkeys that had been inseminated with pooled semen.

MATERIALS AND METHODS

Study 1. Seven commercial turkey farms were scheduled for semen sampling and evaluation of the presence of *Campylobacter* on a weekly basis over a 13-wk period (July to October 2003). However, because of the dynamics of commercial production facilities, which are influenced by management and marketing demands, not all farms were able to provide a weekly sample during the 13-wk period. Semen samples were randomly collected by abdominal massage (5) from four to six toms per farm (45–55 wk of age), aspirated into sterile test tubes, and pooled by farm. Five hundred microliters of pooled raw semen was diluted with 4.5 ml of *Campylobacter*-enrichment broth (CEB), 10-fold serial dilutions were prepared, and 0.1 ml of each dilution was plated onto *Campy*-Line agar (CLA) plates (23). Plates were incubated at 42 C for 48 hr in a microaerophilic environment (5% O_2, 10% CO_2, and 85% N_2). After incubation, characteristic colonies were confirmed as *Campylobacter* by observation of typical cellular morphology using a phase contrast microscope and with a commercial latex agglutination kit specific for *C. jejuni*, *C. coli*, and *C. laridis* (PanBio, Inc., Columbia, MD). The colonies on each CLA plate were counted on a Leico Darkfield Plate Colony Counter (Leico, Inc., Buffalo, NY), and the direct counts were converted to colony-forming units per milliliter (cfu/ml) of pooled semen.

Study 2. Eleven breeder hens (55–57 wk of age) and 18 breeder toms (55–57 wk of age) were randomly selected from four local commercial turkey farms. The turkeys were euthanatized by cervical dislocation and each carcass was placed on its back and the abdominal cavity opened aseptically. Limited necropsies to remove the reproductive tract without contamination from blood and other tissues were carried out. The tracts were then aseptically divided into the appropriate segments (female: vagina, shell gland, isthmus, magnum, and infundibulum; males: testes and ductus

Table 1. Prevalence of *Campylobacter* (cfu/ml) in pooled semen samples of commercial turkey toms 45–55 wks old.[A]

Week	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6	Farm 7
1	NA[B]	NA	2.1×10^3	2.8×10^3	ND[C]	2.0×10^3	2.6×10^3
2	NA	NA	6.3×10^3	1.9×10^4	7.5×10^3	1.8×10^4	2.4×10^3
3	1.5×10^3	NA	1.2×10^3	1.6×10^4	8.3×10^2	1.3×10^3	1.4×10^3
4	1.3×10^3	NA	6.2×10^2	7.5×10^3	1.4×10^2	NA	NA
5	6.7×10^2	NA	3.3×10^2	3.1×10^3	ND	9.0×10^3	NA
6	2.4×10^3	NA	1.8×10^3	3.9×10^2	7.4×10^2	2.1×10^4	NA
7	7.5×10^4	NA	2.8×10^4	9.0×10^5	2.0×10^2	5.1×10^4	NA
8	6.9×10^4	1.5×10^4	NA	6.3×10^4	3.4×10^4	2.4×10^4	NA
9	1.8×10^3	1.8×10^3	3.0×10^3	8.4×10^2	NA	NA	NA
10	1.6×10^6	9.0×10^3	1.6×10^3	3.3×10^4	NA	6.6×10^3	NA
11	4.9×10^3	7.5×10^3	8.1×10^2	1.6×10^2	NA	4.1×10^3	NA
12	NA	2.7×10^3	9.2×10^2	NA	NA	1.5×10^4	NA
13	NA	1.2×10^4	1.8×10^4	NA	NA	NA	NA

[A]Semen samples were collected on a weekly basis over a 13-wk period (July to October 2003) from 4–6 toms/farm, aspirated into sterile test tubes, and pooled by farm. Five hundred microliters of pooled raw semen was diluted with 4.5 ml of CEB, 10-fold serial dilutions were prepared, and 0.1 ml of each dilution was plated onto CLA plates for enumeration of *Campylobacter*.

[B]NA = Not available. Not all farms were able to provide samples during the experimental period.

[C]ND = Not detected.

deferens). Each segment was incised longitudinally and its mucosal surface was swabbed with a dry cotton sterile swab. The swabs were placed in 5.0 ml of CEB and incubated for 24 hr at 42 C in a microaerophilic environment. After incubation, 0.1 ml of the enriched sample solution was streaked onto CLA plates, incubated, and characteristic colonies were confirmed as *Campylobacter* as previously described.

RESULTS

Study 1. *Campylobacter* was isolated from 96.6% (57/59) of the pooled semen samples collected from July to October 2003. *Campylobacter* levels in these pooled semen samples averaged 5.3×10^4 cfu/ml of pooled semen and ranged from below the limit of detection ($<10^1$) to 1.58×10^6 cfu/ml of pooled semen (Table 1). The two pooled semen samples with *Campylobacter* levels below the limit of detection ($<10^1$ cfu/ml) both originated from farm 5.

Study 2. *Campylobacter* was isolated from 10 of the 11 (90.9%) female reproductive tracts collected (Table 2). Of the 11 hens sampled, the vagina displayed the highest number of positive samples (10/11 or 90.9%), followed by the isthmus (8/11 or 72.7%), the shell gland (7/11 or 63.6%), the magnum (6/11 or 54.5%), and the infundibulum (4/11 or 36.4%).

Campylobacter was isolated from both segments (ductus deferens and testes) of the male reproductive tracts that were collected (Table 3). Of the 17 toms sampled, 47.1% (8/17) of the ductus deferens tested positive for *Campylobacter*, while 11.8% (2/17) of the testes were positive.

DISCUSSION

Introduction of *Campylobacter* into a poultry flock by any source, whether by horizontal or vertical transmission, could lead to rapid dissemination within the flock (24). Any successful strategy to reduce or eliminate *Campylobacter* in poultry production systems will require a multifaceted approach and a better understanding of the pathways involved in *Campylobacter* contamination. The colonization of poultry flocks by *Campylobacter* has been thought to derive mainly from horizontal transmission routes and, as a result, intervention strategies have focused on these pathways.

Recently, studies have identified another potential source of *Campylobacter* contamination—vertical transmission of *Campylobacter* from the breeder hen to the offspring (11). Using *Campylobacter* isolates characterized by short variable region (SVR) *flaA* DNA sequences, Cox and coworkers (11) were able to establish evidence of clonal origin between

Table 2. Isolation of *Campylobacter* after enrichment (+ or −) for 24 hr from segments of the reproductive tracts of commercial breeder hens (55–57 wk old).[A]

Hen	Infundibulum	Magnum	Isthmus	Shell gland	Vagina
1	−	−	+	−	+
2	−	−	−	+	+
3	−	+	+	+	+
4	+	+	+	+	+
5	−	+	+	+	+
6	−	−	−	+	+
7	+	+	+	+	+
8	+	+	+	−	+
9	−	−	+	−	+
10	−	−	−	−	−
11	+	+	+	+	+
No. positive	3/11	6/11	8/11	7/11	10/11

[A]The reproductive tracts of 11 randomly selected commercial breeder hens were aseptically excised and divided into the vagina, shell gland, isthmus, magnum, and infundibulum. Each segment was incised longitudinally and its mucosal surface was swabbed with a dry cotton sterile swab. The swabs were placed in 5.0 ml of CEB and incubated for 24 hr in a microaerophilic environment. After incubation, 0.1 ml of the enriched sample solution was streaked onto CLA plates, incubated, and characteristic colonies were confirmed as *Campylobacter*.

Table 3. Isolation of *Campylobacter* after enrichment (+ or −) for 24 hr from segments of the reproductive tract of commercial breeder toms (55–57 wk old).[A]

Tom	Testes	Vas deferens
1	−	+
2	−	+
3	−	+
4	+	+
5	−	+
6	−	−
7	−	−
8	−	−
9	−	+
10	−	−
11	−	+
12	−	−
13	−	−
14	−	−
15	+	+
16	−	−
17	−	−
No. positive	2/17	8/17

[A]The reproductive tracts of 18 randomly selected commercial toms were aseptically excised and divided into the testes and ductus deferens. Each segment was incised longitudinally and its mucosal surface was swabbed with a dry cotton sterile swab. The swabs were placed in 5.0 ml of CEB and incubated for 24 hr in a microaerophilic environment. After incubation, 0.1 ml of the enriched sample solution was streaked onto CLA plates, incubated, and characteristic colonies were confirmed as *Campylobacter*.

breeder hens and commercial broiler flocks that were housed 20 miles apart.

Salmonella, another foodborne pathogen, can also be transferred from parent flocks to their progeny through the transovarian route (26,30), and semen is thought to serve as the vehicle for transmission to the hen and subsequent eggs (27). Because semen on commercial turkey farms, and some commercial broiler breeder facilities, is routinely pooled and then used to inseminate multiple hens, it may be a critical source of *Campylobacter* contamination in the female reproductive tract and fertile eggs.

Bacterial contamination is highly prevalent in poultry semen (26), with reports of an average of 2.2 million bacteria/ml in chicken semen (32) and 1.3 billion bacteria/ml in turkey semen (18). The most frequently isolated bacteria in chicken semen have been *Escherichia*, *Staphylococcus*, *Micrococcus*, *Enterococcus*, and *Salmonella* (26). *Campylobacter* has also been recently isolated in the semen of commercial broiler breeder roosters (12,13) and commercial toms (16). In previous studies using broiler breeder roosters, *Campylobacter* was only isolated from 4.9% of the ductus deferens sampled, 25% of the individual semen samples collected, and 54.5% of the pooled semen samples collected (12,13). In the present study, *Campylobacter* was isolated from the majority (96.6%) of the pooled semen samples collected and from 47.1% of the ductus deferens and 11.8% of the testes. The higher incidence of *Campylobacter* in the pooled semen samples, compared with segments of the male reproductive tract in the present study with turkeys and previous studies with roosters (12,13), indicates that mixing semen from uninfected and infected males results in an increase in the prevalence of contaminated pooled semen. Furthermore, it is possible that the semen may have become contaminated with enteric *Campylobacter* during the collection process. This idea is supported by early research demonstrating that semen collection is predisposed to fecal contamination because of the tom's anatomy (22,31).

Although *Campylobacter* has been isolated in all sections of the female chicken's reproductive tract (4) and in chicken eggs (7,28), there are no reports of *Campylobacter* detection in the reproductive tracts of turkeys or turkey eggs. In the present study, *Campylobacter* was isolated from all segments of the female turkey reproductive tract. Overall, the incidence of *Campylobacter* was higher toward the lower portion of the reproductive tract (vagina), closest to the cloaca. However, it was interesting that *Campylobacter* was observed in several hens as far up the tract as the infundibulum.

In total, these data demonstrate that turkeys, like chickens, have a significant incidence of *Campylobacter* infection in the reproductive tract and pooled semen. These data also support the possibility that *Campylobacter* is vertically transferred in turkeys, as is the case in chickens (11). This may be an important finding, as commercial turkey production relies on artificial insemination, and the random pooling of semen may be a source of *Campylobacter* contamination. In an effort to reduce the potential for contamination, our laboratory has tried to eliminate *Campylobacter* in turkey semen by using commercially available semen extenders containing various combinations of antibiotics (16) or by altering the temperature and oxygen environments (9), which may attenuate *Campylobacter* survival *in vitro.* These studies have failed to significantly reduce *Campylobacter* concentrations in semen. Additional studies will be needed to devise strategies to eliminate *Campylobacter* contamination in semen and the reproductive tracts of poultry.

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